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Establishment of a culture condition for strong proliferation and enrichment of chicken spermatogonial stem cells in vitro

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Keywords

spermatogonial stem cell; Gallus gallus; proliferation; growth factors; B27

Abstract

Poultry spermatogonial stem cells (SSCs) have the potential to serve as a model for studying the basic biology of SSC and they can also be used for biotechnological purposes. However, the small number of SSCs and the presence of the testicular somatic cells with SSCs have limited their applications. Therefore, this study was undertaken for the first time to investigate the effect of a serum-free medium supplemented with a combination of specific growth factors and B27 on the proliferation and enrichment of newborn chicken SSCs in vitro. Newborn chicken testicular cells were cultured in a serum-free DMEM, supplemented with GDNF, bFGF, LIF, and EGF growth factors and also B27 as an alternative for FBS. Presence and maintenance of the SSCs in the enriched cultures were evaluated by detection of alkaline phosphatase (AP) activity and ASZ1, POU5F1, CVH and GPR125 gene expression. A small number of clusters and colonies were emerged in testicular cell cultures before treatment with the enriched cell culture medium. Enrichment of the DMEM with the above indicated factors strongly promoted the proliferation of the chicken SSCs. Moreover, this culture condition declined attachment and maintenance of the testicular somatic cells and thus they decreased gradually in the cultures. The enriched SSCs were positive for AP activity and with detectable levels of ASZ1, POU5F1, CVH and GPR125 gene expression. This study shows that serum-free medium supplemented with a combination of B27 and the above indicated growth factors induces proliferation and enrichment of chicken SSCs in vitro in a short period of time.

Abbreviations

AP: Alkaline Phosphatase
ASZ1: Ankyrin Repeat, Sam And Basic Leucine Zipper Domain Containing 1
BFGF: Basic Fibroblast Growth Factor
CVH: Chicken Vasa Homologue
CSF-1: Colony Stimulating Factor 1
DMEM: Dulbecco's Modified Eagle's Medium

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EGF: Epidermal Growth Factor
FBS: Fetal Bovine Serum
GSCs: Germ Stem Cells
GDNF: Glial Cell Line-Derived Neurotrophic Factor
GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase
GPR125: G-Protein Coupled Receptor 125
IGF1: Insulin-Like Growth Factor 1
LIF: Leukemia Inhibitory Factor
POUF1: Pou Domain, Class 5, Transcription Factor 1
RT-PCR: Reverse Transcription-PCR
STO: SIM Thioguanine/Ouabain-Resistant Mouse Fibroblast Cell Line
SSCs: Spermatogonial Stem Cells

Introduction

Spermatogonial stem cells, also known as germ stem cells (GSCs), are the basis of continual spermatogenesis in testes of adult male organisms (Zohni et al., 2012). There is special interest for derivation of chicken (*Gallus gallus*) SSCs, as an important tool for studying cell and molecular biology of GSCs and more importantly they can be used in biotechnology for production of transgenic chickens as bioreactors to produce some recombinant industrial and medical proteins (Li & Lu, 2010; Yu et al., 2010). Despite the substantial progress in culture and induction of self-renewality, expansion, and enrichment of SSCs from mammals during the past decade, there are not many such reports for chicken SSC populations in vitro.

Efforts have been made to develop special culture conditions for induction of proliferation of the mammalian SSCs in vitro in order to provide sufficient numbers of SSCs for different applications. Different studies have revealed that glial cell line-derived neurotrophic factor (GDNF), leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF), insulin-like growth factor 1 (IGF1), epidermal growth factor (EGF), and colony stimulating factor 1(CSF-1) are required for self-renewal and expansion of mammalian stem cells both in vivo and in vitro (Oatley & Brinster, 2008; He et al., 2009). Moreover, B27 had originally been optimized for culture and growth of neural progenitor or stem cells (Brewer, 1995). A previous study showed that replacement of the serum in medium with B27, minus vitamin A supplement, attenuated the proliferation of somatic cells in vitro by their gradual removal from the culture (Hamra et al., 2005). B27 supplement, as an alternative for fetal bovine serum (FBS), contains vitamins like biotin, DL-alpha-tocopherol, and DL-alphatocopherol acetate and other components such as human recombinant insulin, D-galactose, catalase, reduced glutathione linoleic acid, linolenic acid, superoxide dismutase, ethanolamine hydrochloride, triiodo-L-thyronine, corticosterone, etc (Chen et al., 2008; Krishnan et al., 2010). There is no reported study related to the effects of serum-free medium supplemented with the combination of specific growth factors (GDNF, bFGF, LIF, and EGF) and B27 on expansion and enrichment of chicken SSCs in vitro. Thus, this study was designed to investigate the effect of this culture condition on proliferation and enrichment of newborn chicken SSCs in vitro.

Materials and Methods

Experimental animals

White Hy-line W36 newborn chickens, were obtained from the Morghak Co., Mashhad, Iran. The guidelines of Bioethics committee of the Ferdowsi University of Mashhad were followed for animal management and surgery.

Testicular cell culture

Derivation of testicular cells, which include SSCs from chicken testes, was performed according to our established protocol (Momeni-Moghaddam et al., 2014). Briefly, after isolation of chicken testes (from 5 animals), they were cut into small pieces mechanically using a scalpel. The specimens were then cultured in a 12 well plate in the presence of Dulbecco's modified Eagle`s medium (DMEM) (Gibco, Scotland) containing 10% (v/v) FBS (Gibco, Scotland), and 1x penicillin-streptomycin (PAA, Austria) at 37ºC and 5% CO_2. After 48 h the tissue fragments were removed and fresh medium was added to cover the detached single cells, released from the specimens. Finally, confluent wells (after 72 h) were trypsinized (using 0.25% trypsin) and collection of the cells from two wells were transferred into a T25 flask and incubated under the same conditions. The presence of aligned cells, clusters, and colonies, as indication of SSCs derivation, were investigated using an inverted microscope (hp, China).

Expansion and enrichment of SSC populations

For expansion and enrichment of chicken SSCs, we used differential trypsinization (0.25% trypsin) of the total testicular cells at passage one, followed by filteration, using 30 µm pre-separation filters (Miltenyi Biotec, Auburn, CA, USA). Pre-separation filters are designed for the easy removal of cell clumps or large particles from the single-cell suspensions. Cell clumps and also SSC colonies, which were not dissociated, remained on the surface of the filter and single cells passed through it and they were collected in a falcon tube. To examine the effect of an enriched cell culture medium on SSCs proliferation in vitro, we were provided four groups of cell cultures, including the cultures from the dissociated cell clumps and colonies fraction (T1) and single cell fraction which was passed though filter (T2) in a serum-free medium supplemented with B27 and specific growth factors, in comparison with their untreated counterparts as controls (C1 and C2, respectively). To provide T1 cell culture, cell clumps and SSC colonies were collected and dissociated by vigorous pipetting and cultured in 6 well plates in a seeding density of 0.15×10^6 cells/well in the presence of serum-free DMEM, supplemented with

Table 1
Primer sequences used in gene expression analysis by RT-PCR.

Primer name	Primer sequence	Product size
F-GAPDH	5`- CCTTCATCGATCTGAACTACATGG -3`	265 bp
R-GAPDH	5`- GGAGCTGAGATGATAACACGCTTA -3`	
F-ASZ1	5`- TGGACAGAGGTGCTAATGC -3`	145 bp
R-ASZ1	5`- CTGCAAGTAAGGTTAGGGTC -3`	
F-POU5F1	5`- AATGAGGCAGAGAACACGGACAAC -3`	154 bp
R-POU5F1	5`- GGGACTGGGCTTCACACATTTGC -3`	
F-CVH	5´- CAGGCGTGGATGGCTAACTC -3´	274 bp
R-CVH	5´- CAGAACTCCTCCCTCTACCAAATC -3´	

B27 (0.004%) (Gibco, Scotland), bFGF (20 ng/ml) (Sigma, Germany), LIF (20 ng/ml) (Sigma, Germany), EGF (20 ng/ml) (Sigma, Germany), and GDNF (15 ng/ml) (Sigma, Germany). Moreover, the collected media, containing the single cells in the falcon tubes, were centrifuged (5 min at 70 g) and their cell pellets were resuspended in enriched serum-free DMEM containing factors as indicated before to provide T2 cell culture. The resuspended cells were also cultured in 6 well plates at conditions similar to cell clumps and colonies at 37°C in a humidified atmosphere with 5% CO_2. Furthermore, untreated cultures containing DMEM supplemented with 10% FBS were used as controls (C1 and C2 cell cultures) to ensure reliable results. Every day, the cell cultures were assessed microscopically and the number of colonies in the treated cultures and untreated counter parts were recorded.

SSC characterization

Alkaline phosphatase staining

Colony-containing chicken testicular cells were fixed with 4% paraformaldehyde solution (for 12 min) and then rinsed with Phosphate Buffered Saline (PBS) for three times to remove fixation solution. For staining, the fixed cells were immersed in AP staining solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP 1%)–Nitro blue tetrazolium (NBT 1.5%) (Fermentas, Germany) for 15 min and then rinsed three times with PBS.

RT-PCR analysis

Total RNAs were extracted using Trizol reagent (BioNeer, Korea) from the enriched colony-containing and also non colony-containing chicken testicular cell cultures. The quality of the extracted RNAs were verified by agarose (Sigma, USA) gel electrophoresis and then RNAs were treated with DNase I (Fermentas, Germany) to remove possible contaminating genomic DNA. The DNase I treated RNAs (1 µg of each total RNA) were reverse transcribed into cDNA using RT-enzyme (MMLV; Fermentas, Germany) at 42°C for 60 min. The quality of the resulting cDNAs was determined by PCR analysis for expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as housekeeping gene. Then, PCR reaction was performed for 40 cycles using specific primers (Table 1) in an annealing temperature of 58°C for ASZ1 (GASZ), POU5F1 (OCT4/cPOUV) and CVH genes. The amplification products were visualized by electrophoresis on 1.5% agarose gel and ethidium bromide staining.

Immunocytochemical analysis

Immunocytochemistry assay was used for detection of POU5F1 and GPR125 positive cells in enriched cultures. Briefly, colony-forming cells were fixed in 4% paraformaldehyde solution for 12 min and then rinsed with PBS for 5 min to remove fixation solution. Cellular permeabilization was achieved by treatment with 0.5% Triton X-100 for 10 min in order to detect OCT4 protein. After washing with PBS, 3% H_2O_2 in PBS was used for 30 min to block the endogenous peroxidases. To minimize nonspecific binding, the permeabilized cells were treated with 4% bovine serum albumin (BSA) (for 45 min) before immunostaining. The optimal concentration of each antibody was selected based on preliminary experiments. These concentrations were as follows: 1:50 for mouse anti-human OCT4 (Santa Cruz, Germany, Cat. No. sc-5279) and 1:200 for rabbit anti-human GPR125 (Abcam, UK, Cat. No. ab51705) (Sisakhtnezhad et al., 2015) (this antibody identified an epitope of chicken GPR125 with 99% similarity to human GPR125 epitope). After treatment for 2 h with the primary antibodies, the cells were incubated with secondary antibodies including IgG2b-horseradish peroxidase (HRP) to anti-OCT4 antibody (1:500; R & D Systems, USA, Cat. No. ab97250) and IgG-FITC to anti-GPR125 antibody (1:100; Razi BioTech, Iran, Cat. No. AF8035) for 50 min. For OCT4 immunocytochemistry, after removing the secondary antibodies, the cells were sequentially subjected (for 5 min) with a chromogenic substrate of HRP enzyme (diamino-benzidine (DAB) working solution) (Fermentas,

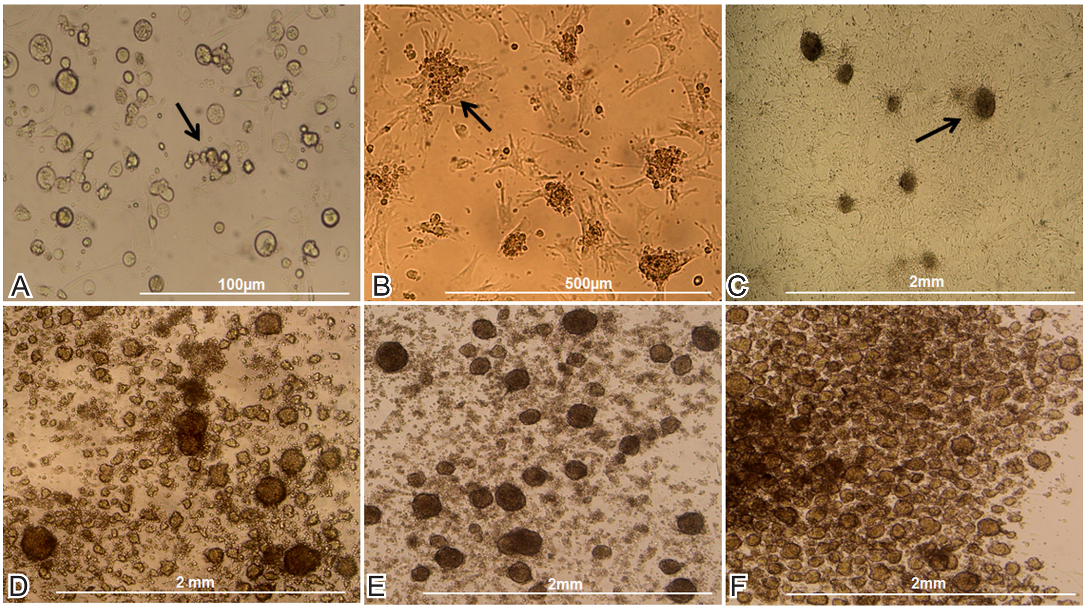


Figure 1
Culture and enrichment of chicken SSC populations in vitro. The appearance of aligned (A), clusters (B) and colonies (C) of SSCs in chicken testicular cell cultures are indicated by arrows. A few numbers of colonies were produced in the chicken testicular cell culture in serum containing DMEM. Clusters and colonies resulted from cultivation of SSC colonies and cell clumps in serum-free DMEM, supplemented with B27 and four growth factors (GDNF, bFGF, LIF, and EGF), on untreated wells were developed (D). Most of the clusters and colonies were floated at day 1. These clusters and colonies grew in size and mostly attached to the surface of the plate at day 2 (E). Cultivation of single cells, passed through pre-separation filters, in serum free DMEM supplemented with B27 and four growth factors (GDNF, bFGF, LIF and EGF) on untreated wells resulted in production of hundreds of floated clusters and colonies at day 1 (F).

Germany) and then observed under an inverted microscope for presence of brown color. In case of GPR125, after washing and removal of unattached secondary antibodies, the cells were observed under a fluorescent microscope (hp, China) for presence of green fluorescence marker. Negative controls, without primary antibodies, were used to check for production of background color during immunostaining.

Statistical analysis

Statistical analysis of the colony formation in cultures was carried out by one-way analysis of variance and Tukey`s test in SPSS software (version 16). All data were expressed as mean±SD and the level of $P<0.05$ was used as a criterion of statistical significance.

Results

Derivation of testicular cells

Cells from the fragmented testes began to attach to the surface of the plates 24 h after culture. The excessive fragments were removed after 48 h, when a few clusters and small colonies had emerged in these primary cultures. The culture plates reached confluency 3-4 days after culture. After the first passage, clusters and colonies expanded and the frequency of clusters and colonies was at least ten times higher than that in the primary cultures (Figure 1A-C). As show in Figure 1B and D, the testicular somatic cells act as a feeder layer for attachment and maintenance of the clusters and colonies.

Expansion and enrichment of chicken SSCs in culture

Total chicken testicular cell culture was trypsinized by short time incubation with trypsin in order to differentially detach and filter- or separate the colonogenic cells from the single cells. The proliferation rates of the two fractions were examined in the enriched cell culture media in the culture plates.

Cultivation of the cell clumps and colonies fraction in the enriched medium induced proliferation of the colony-forming cells one day after culture, with production of numerous floated small clusters and sub-colonies (Figure 1D). These newly formed small colonies became larger in size after 2 days and were attached to the surface of the wells (Figure 1E). As shown in Figure 2, the rate of colony formation in treated wells indicates a 10-fold increase in comparison with untreated counterparts at the same time point (72h after treat). In this culture condition, the testicular somatic cells population in enriched cultures were mainly floated and removed when the cell's culture medium was replaced with fresh medium. Therefore, decrease of the testicular somatic cells which act as a feeder layer for the colonies and extreme growth of the size of colonies, induced death and gradual removal of the colonies from the cultures. The enriched cultures were maintained up to 3 weeks after the initial culture and they were passaged each 4 days.

Similar results were obtained for the collected single

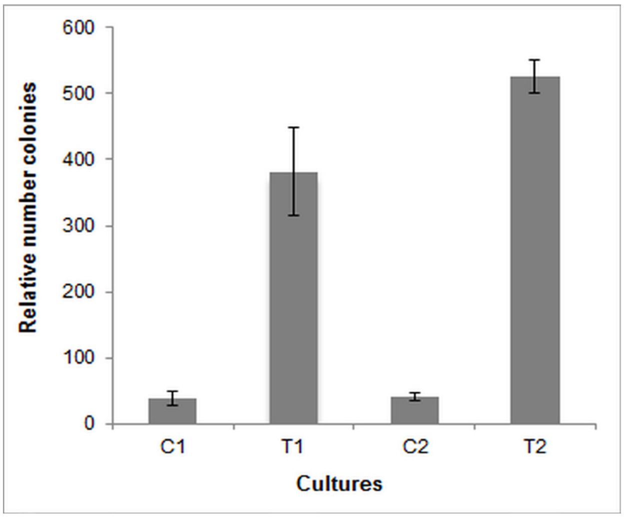


Figure 2
Statistical analysis of the colony formation in cultures. Colony-forming efficiency is affected by cultivation of the cell clumps and colonies fraction (T1) and single cell fraction which was passed though filter (T2) in a serum-free medium supplemented with B27 and specific growth factors, in comparison with their untreated counterparts as control (C1 and C2, respectively). Results are represented as means ± SD of three repeats.

cells, which were passed through the filter, when cultivated in the presence of an enriched medium on plates (Figure 1F). As show in Figure 2, the number of colonies increased 12.5-fold after using the enriched cell culture media in contrast to the control cell culture, containing DMEM supplemented with 10% FBS. Overall, culture of the collected single cells in the enriched media resulted in increased number of colonies in comparison to cultivation of the cell clumps and SSC colonies in similar conditions.

Characteristics of the derived SSCs

RT-PCR analyses

RT-PCR results demonstrated that the investigated genes of ASZ1, POU5F1 and CVH were expressed in the enriched colony-containing chicken testicular cell culture, while they were not detectable in the culture without cellular colonies (Figure 3). Thus, these results indicate that ASZ1, POU5F1 and CVH are specific markers of colony-forming cells in chicken testicular cell cultures. The PCR product size for each gene is shown in Table 1.

Alkaline phosphatase activity

One of the criteria to confirm the cell as self-renewing SSCs is their positive AP activity. The enriched cell cultures from this study included many single cells, clusters, and colonies with detectable levels of AP activity (Figure 4A, B). AP activity was revealed as dark violet color in single colony-forming cells and resulting colonies. Moreover, from each enriched culture, a few cells were noticed without detectable AP activity (Figure 4B).

Immunocytochemistry analyses

Immunocytochemistry studies with anti-POU5F1 and anti-GPR125 antibodies were also performed on the enriched cultures for determination of SSCs identity of the expanded colony-forming cells. The detected single colony-forming cells, clusters, and colonies from the enriched cultures, unlike other chicken testicular cells in culture, were expressing the POU5F1 (Figure 4D-F) and GPR125

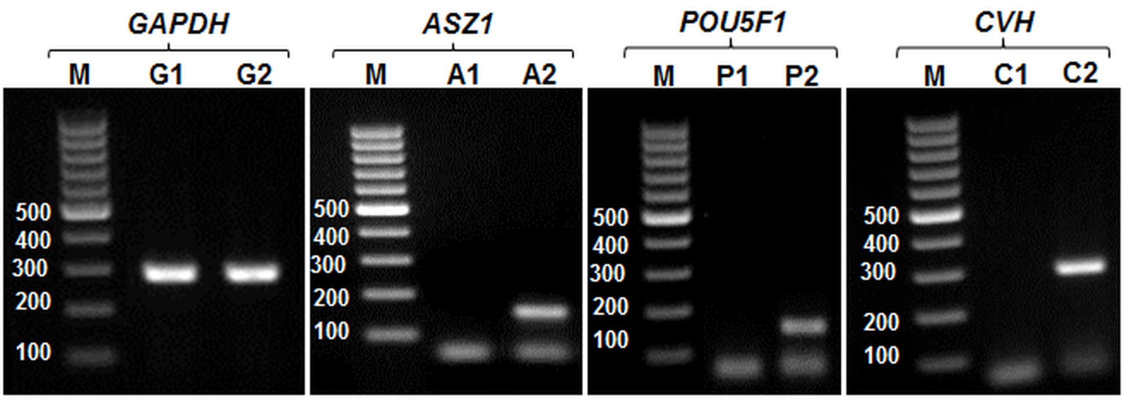


Figure 3
Gene expression analysis of SSCs specific genes at mRNA level. RT-PCR analysis for GAPDH, ASZ1, POU5F1 and CVH mRNA expression in chicken testicular cell culture without colonies (G1, A1, P1, C1, respectively) and enriched cell culture (G2, A2, P2, C2, respectively). ASZ1 (A1), POU5F1 (P1) and CVH (C1) were not expressed in chicken testicular cell culture without colonies. Expression of ASZ1 (A2), POU5F1 (P2), and CVH (C2) were detected in the enriched cell culture at mRNA level. (M) represents 100 bp DNA ladder.

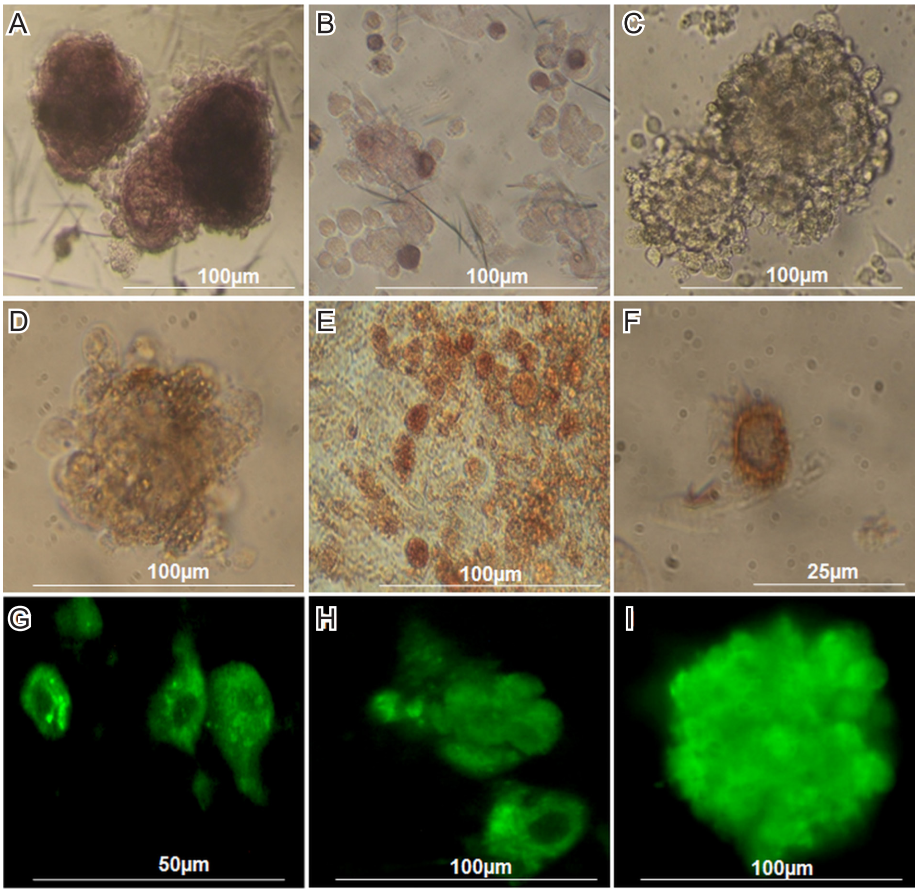


Figure 4
Alkaline phosphatase activity and immunocytochemistry analyses. Alkaline phosphatase assay and immunocytochemistry analysis of POU5F1 and GPR125 expression for verification of the cells with SSC characteristics in the enriched cell cultures at passage 3. AP activity of the SSC colonies (A) and also single colony-forming cells (B), can be observed. Micrograph (C) shows morphology of the enriched SSCs before AP assay. Immunocytochemical analysis indicated that POU5F1 was expressed in SSC colonies (D) and single colony-forming cells (E, F). Moreover, GPR125 was expressed in the single colony-forming cells (G), clusters (H), and colonies of SSCs (I).

markers (Figure 4G-I).

Discussion

Despite the great potentials of SSCs for elucidation of the basic biology of developmental cascades in mammals and also their substantial implications in regenerative medicine, drug discovery, and biotechnological disciplines, the small number of SSC populations in testis tissue has rendered disappointment for achieving these potentials so far. Development of simple methods for derivation, characterization and also induction of self-renewality, expansion, and enrichment of the SSCs is critical for providing a sufficient number of them. Thus, this study was conducted to develop a simple culture method to assess large number of chicken SSCs for desired applications. In this study, mechanical digestion was employed for access to the testicular cells including spermatogonia in newborn chicken testes. This method was used as a simple, efficient, and routine practice for derivation of SSCs from chickens in our laboratory (Momeni-Moghaddam et al., 2014). Different investigations have demonstrated that the preliminary criterion to confirm the presence of chicken SSCs in the testicular cell cultures is detection of aligned cells, or clusters, and colonies of them during continuous mitotic division (Jung et al., 2010; Yu et al., 2010; Sisakhtnezhad et al., 2015). Accordingly we followed the same notion and confirmed that presence of these colony-forming cells was also evident in our cultures, derived from testicular tissues of the newborn chickens, although their population was limited in the preliminary culture conditions.

There are limited numbers of studies claiming different specific growth requirements for chicken SSCs. These studies demonstrated that DMEM, supplemented with fetal calf serum (FCS), chicken serum, L-glutamine, gentamicin sulfate, sodium pyruvate, β-mercaptoethanol, non-essential amino acids, SCF, LIF, bFGF, IL-11, and IGF are required for chicken SSC self-renewality and survival in vitro in the presence of a feeder layer (Jung et al., 2007; Li et al., 2008; Yu et al., 2010). Moreover, our previous study has shown that GDNF, bFGF, and LIF could induce proliferation of chicken SSCs and GDNF was more influential factor (Momeni-Moghaddam et al., 2014). However, several studies on mammalian SSCs have indicated that the effects of each growth factor alone are very limited (Kanatsu-Shinohara et al., 2003; Kanatsu-Shinohara et al., 2005; Kanatsu-Shinohara et al., 2006). In this study, DMEM medium was supplemented by a combination of four different growth factors (GDNF, bFGF, LIF, and EGF) and also B27 as an alternative for FBS. The optimal concentration of each growth factor was selected based on previous experiments in our laboratory. Although B27 had originally been optimized for culture and growth of mammalian neural progenitor or stem cells (Brewer, 1995), it was also used as an alternative of serum for culture of other cells. The results of the present study demonstrated that the enriched serum-free DMEM medium, supplemented

with B27, GDNF, bFGF, LIF, and EGF strongly promotes proliferation of the chicken SSCs in contrast to the control cell cultures. The number of colonies was increased several times against the control cell cultures, 72 h after the initial culture. Therefore, it can be claimed that induction of the proliferation and enrichment of the chicken SSCs using DMEM supplemented with the indicated factors in a short period of time, and also without feeder layer such as STO or synthetic matrix are major progresses made in the current study, in comparison with previous studies (Jung et al., 2007; Momeni-Moghaddam et al., 2014).

In this study, the identity of SSCs in the enriched cell cultures was confirmed by AP assay, RT-PCR and immunocytochemistry analyses. Studies on SSCs from different organisms such as chicken have indicated that SSCs are alkaline phosphatase positive cells (Huang et al., 2009; Yu et al., 2010; Sisakhtnezhad et al., 2015). The results of this study also indicate that the enriched colony-forming cells were showing AP activity. In addition, Yan and colleagues demonstrated that ASZ1 is a specific marker of mouse germ cells which is not expressed in other cells and tissues (Yan et al., 2002). Also, SSCs as germ cell subpopulations in testis have proven to express POU5F1 (as a pluripotent marker) in mouse SSCs (Oatley et al., 2006; Huang et al., 2009). Moreover, CVH (chicken VASA homology) is required for specification of germ cell lineage in chickens (Lavial et al., 2009). Eildermann and colleagues demonstrated that VASA is a specific biomarker of primate SSCs and used these markers for discrimination of SSCs from testicular multipotent stromal cells in vitro (Eildermann et al., 2012). Furthermore, based on previous studies in other organisms GPR125 has been considered as a reliable biomarker for characterization of mammalian SSCs (Seandel et al., 2007; He et al., 2010; Sisakhtnezhad et al., 2015). Expression of these four important markers in the colony-forming cells was indicative of their SSC identity. The results of this study also demonstrated that the enriched colony-forming cells were positive for ASZ1, POU5F1, CVH and GPR125.

In conclusion, this study provides a simple culture condition, in a short period of time, for expansion and enrichment of newborn chicken SSCs, allowing for production of large numbers of SSCs which could be used for studying the basic biology of SSCs and application in biotechnological and transgenesis programs. We report that use of serum-free medium supplemented with B27, GDNF, bFGF, LIF, and EGF strongly promotes expansion and enrichment of the chicken SSCs in vitro. Moreover, chicken SSC populations kept their characteristics during this culture condition.

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چکیده

سلول‌های بنیادی اسپرم‌ساز پرندگان پتانسیل بالقوه‌ای به عنوان مدل برای مطالعات پایه‌ای زیست‌شناسی سلول‌های بنیادی اسپرم‌ساز و نیز استفاده برای اهداف بیوتکنولوژی دارند. با این حال تعداد اندک این سلول‌ها و حضور سلول‌های سوماتیک بیضه‌ای در کنار سلول‌های بنیادی اسپرم‌ساز، کاربردهای این سلول‌ها را محدود نموده است. بنابراین، این مطالعه برای اولین بار، به منظور بررسی اثر یک محیط کشت فاقد سرم و غنی شده با ترکیبی از انواع فاکتورهای رشد و نیز B27، بر تکثیر و غنی‌سازی جمعیت سلول‌های بنیادی اسپرم‌ساز جوجه تازه متولد شده تحت شرایط آزمایشگاهی، انجام شد. برای این منظور سلول‌های بافت بیضه جوجه تازه متولد شده در یک محیط کشت فاقد سرم و غنی شده با فاکتورهای رشد GDNF، bFGF، LIF و EGF و همچنین B27 به عنوان جایگزینی برای سرم، کشت داده شدند. حضور سلول‌های بنیادی اسپرم‌ساز در کشت‌های سلولی حاصل از بافت بیضه بوسیله تشخیص فعالیت آنزیم آلکالین فسفاتاز و بیان ژن‌های ASZ1، POU5F1، CVH و GPR125 مورد بررسی قرار گرفت. قبل از تیمار با محیط کشت غنی شده، تعداد اندکی از خوشه‌ها و کلونی‌های مرتبط با سلول‌های بنیادی اسپرم‌ساز در کشت‌های سلولی ظاهر شدند. غنی شدن محیط کشت DMEM با فاکتورهای ذکر شده در بالا، شدیدا منجر به القاء تکثیر سلول‌های بنیادی اسپرم‌ساز جوجه شد. بعلاوه، تحت این شرایط کشت، اتصال و ماندگاری سلول‌های سوماتیک بیضه‌ای کاهش یافت و بنابراین به تدریج تعداد آنها در محیط کشت کاهش یافت. سلول‌های بنیادی اسپرم‌ساز غنی شده برای فعالیت الکالین فسفاتاز و بیان ژن‌های ASZ1، POU5F1، CVH و GPR125 مثبت بودند. این مطالعه نشان می‌دهد که محیط کشت غنی شده با فاکتورهای رشد ذکر شده در بالا و نیز B27 باعث القاء تکثیر و غنی‌سازی جمعیت سلول‌های بنیادی اسپرم‌ساز در کوتاه مدت تحت شرایط آزمایشگاهی می‌شود.

واژگان کلیدی: سلول‌های بنیادی اسپرم‌ساز، جوجه، تکثیر، فاکتور رشد، B27